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Hepatoprotective potential of *Azima tetracantha* and *Tribulus terrestris* on ferrous sulfate–induced toxicity in rat

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Abstract

The present study is to evaluate the antihepatotoxic effect of hydroalcoholic extract of leaf powder of *Azima tetracantha* and the fruit powder of *Tribulus terrestris*. Ferrous sulfate was used to induce hepatotoxicity and Silymarin was used as a standard drug. The level of biochemical parameters such as protein, albumin, globulin, HDL, vitamin E, superoxide dismutase and catalase were observed to be decreased and the level of glucose, LDL, VLDL, bilirubin, cholesterol, triglycerides, alkaline phosphatase and TBARS were increased in hepatotoxicity-induced rats. Retrieval of liver parameters to normal level was obtained after the oral administration of herbal drugs. Histopathological studies revealed diminished hepatocellular injury in the herbal drugs treated rats. As a conclusion hydro alcoholic extract of leaf powder of *A. tetracantha* and fruit powder of *T. terrestris* were possesses significant hepatoprotective activity.

Introduction

Liver is the vital organ responsible for drug metabolism and appears to be a sensitive target site for substances modulating biotransformation (Ahmad et al., 2002). Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and auto-immune disorders. Certain medicinal agent introduced within therapeutic ranges may injure the organ some times. Other chemical agents those used in herbal remedies may also induce hepatotoxicity (Boerth et al., 2002). Now-a-days drug induced liver injury has become a major health problem. Liver diseases such as jaundice, cirrhosis and fatty liver are very common and large public health problems in the world (Balamurugan et al., 2008). There is no rational therapy available for treating liver disorders so that management of liver diseases is still a challenge to the modern medicine. The traditional system of medicine has a major role in the treatment of liver ailments.

Azima tetracantha Lam. is a popular herb in Indian traditional medicine used as an antiarthritic, antimicrobial, hepato- and nephro-protective agent. Whole plant extract of *A. tetracantha* contains flavonoids, amino acids, tannins, saponins and alkaloids, which may be responsible for the above activities (Jasulanth et al, 2001). In India and Sri Lanka the root, root bark and leaves are added to food as a remedy for rheumatism. The plant is considered as diuretic, used to treat dropsy, dyspepsia, chronic diarrhea and as a stimulant tonic (Bennett et al., 2004).

Tribulus terrestris is an annual plant native of Mediterranean region. *T. terrestris* is an important herb commonly used as folk medicine in many countries for different purposes. Fruits of *T. terrestris* have been shown to exhibit diuretic, antiurolithiatic, CNS stimulant, antimicrobial and antifungal activities in rats (Tuncer et al., 2009).

Iron overload is most often diagnosed when tissue damage occurs, especially in iron storing organ such as the liver. Hepatic fibrosis and cirrhosis are the outcomes of chronic iron overload due to inherited conditions as well as to repeated blood transfusions (Khan, 2012). Hepatotoxicity manifest as damage or dysfunction of the liver is second only to cardiovascular

collapse as the cause of the death due to acute iron poisoning. An understanding of the pathogenesis of the hepatotoxicity of acute iron poisoning is central to the identification of rational and effective interventions (Firdous, 2012).

Hence the present study was undertaken to investigate hepatoprotective activity of *A. tetracantha* and *T. terrestris* against ferrous sulfate induced toxicity in experimental rats.

Materials and Methods

Animals

Healthy young albino rats weighing 130-150 g were purchased from a animal house, Manapparai, Tamilnadu and were acclimatized for 10 days under standard housing conditions (24 ± 1°C: 45-55%RH with 12 hours light/dark cycle). The animals had free access to rat food and water *ad libitum*. The animals were habituated to lab conditions for two days to the experimental procedures to minimize any non specific stress. The experiment was designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC).

Chemicals

Ferrous sulfate (AR) was purchased from Merck (India). Silymarin (standard drug) was procured from Sigma-Aldrich, Bangalore. Other solvents and chemicals were of analytical grade and purchased locally.

Plant materials

A. tetracantha leaves were collected from Pattukkottai, Tamilnadu and *T. terrestris* fruits were collected from Needamangalam near Thanjavur, Tamilnadu.

Dose preparation and administration

A. tetracantha leaves and *T. terrestris* fruits were dried at 45°C for 48 hours, powdered using electric grinder and stored in a container. Powders (200 g) were extracted with hydroalcoholic mixture (ethanol and water in 1:1 proportion) at room temperature and filtrate were collected and concentrated. 1 mL of filtrate was fed to the animals orally.

Experimental design

Group I**:** Six rats were kept as control; Group II: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st–24th days once daily; Group III: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st–14th days once daily and then administered hydroalcoholic extract of fruit powder of *T. terrestris* on 15th–24th days once daily; Group IV: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st–14th days once daily and then administered hydroalcoholic extract of leaf powder of *A. tetracantha* on 15th–24th days once daily; Group V: Six rats were administrated with 100 mg/kg b.w of ferrous sulfate on 1st–14th days once daily and then administered 300 mg of silymarine on 15 th–24th days once daily.

Biochemical investigation

At the end of treatment rats were sacrificed by cervical decapitation and subjected to various biochemical: sugar (Winckes and Tietz, 1971), bilirubin (Malloy et al., 1937), protein (Lowry et al., 1951), albumin, globulin (Doumas et al., 1971), alkaline phospatase (Tiez, 1983), total cholesterol (Zlatis et al., 1953), triglycerides (Rice, 1970), lipoproteins (Fridwald et al., 1972), TBARS (Fraga et al., 1988), superoxide dismutase (Kakkar et al., 1984), catalase (Sinha, 1972) and vitamin E (Zaspal, 1983) and histopathological (Bancroft, 1977) assays.

Results

Table I shows the levels of sugar, bilirubin, protein, albumin, globulin, A/G and alkaline phospatase. There is a significant increased level of sugar, bilirubin and alkaline phospatase; decreased levels of protein in Group II intoxicated rats. On administration of hydro-alcoholic extracts of herbal drugs and

Table II shows the levels of total cholesterol, triglycerides, HDL, LDL, VLDL, TBARS, superoxide dismutase, catalase and vitamin E. There is a significant increased level of, cholesterol, triglycerides, LDL, VLDL and TBARS decreased levels of HDL, superoxide dismutase, catalase and vitamin E in Group II intoxicated rats. On administration of hydro alcoholic extracts of herbal drugs and standard drug reverse the levels near to Group I rats.

Table I

Changes in the levels of serum sugar, bilirubin, cellular enzyme and protein levels

Parameters	Group I	Group II	Group III	Group IV	Group V
Sugar	90.5 ± 4.6	285.5 ± 4.2[a]	145.0 ± 4.1[b]	128.5 ± 2.9[b]	164.0 ± 7.2[b]
Bilirubin	0.4 ± 0.2	1.6 ± 0.3[a]	1.2 ± 0.2[ns]	0.9 ± 0.2[b]	1.3 ± 0.2[ns]
Protein	6.97 ± 0.2	2.9 ± 0.2[a]	6.6 ± 0.1[b]	6.8 ± 0.3[b]	6.6 ± 0.4[b]
Albumin	3.6 ± 0.2	1.9 ± 0.3[a]	3.1 ± 0.2[b]	3.6 ± 0.3[b]	2.8 ± 0.2[b]
Globulin	2.87 ± 0.1	1 ± 0.4[a]	3.7 ± 0.6[b]	3.1 ± 0.2[b]	3.8 ± 0.4[b]
A/G Ratio	1.4 ± 0.1	2.5 ± 2.1[ns]	0.9 ± 0.2[b]	1.2 ± 0.2[b]	0.8 ± 0.2[b]
Alkaline phosphatase	135.5 ± 2.8	447.0 ± 6.4[a]	210.0 ± 6.3[b]	163.7 ± 3.6[b]	242.5 ± 4.1[b]

Values are expressed in mean ± S.D.; [a]p= Significant different from Group I vs. Group II ($p<0.001$) [b]p= Significant different from Group II vs. Group III, IV and V ($p<0.001$)

Table II

Changes in the levels of serum lipid profile, liver TBARS and liver anti-oxidants

Parameters	Group I	Group II	Group III	Group IV	Group V
Total cholesterol	115.0 ± 3.5	348.2 ± 1.7[a]	190.0 ± 4.5[b]	172.5 ± 2.1[b]	212.5 ± 8.7[b]
Triglycerides	90.0 ± 2.9	200.0 ± 2.1[a]	99.7 ± 2.5[b]	120.5 ± 3.7[b]	110.8 ± 0.4[b]
HDL	47.3 ± 4.6	28.5 ± 2.1[a]	42.8 ± 2.9[b]	45.3 ± 3.3[b]	39.5 ± 3.8[b]
LDL	49.8 ± 8.1	357.7 ± 1.3[a]	127.3 ± 7.0[ns]	103.1 ± 1.7[ns]	150.8 ± 8.7[ns]
VLDL	18.0 ± 0.5	40.0 ± 0.4[a]	19.9 ± 0.5[b]	24.1 ± 0.7[b]	22.1 ± 0.1[b]
TBARS	0.4 ± 0.1	0.8 ± 0.1[a]	0.5 ± 0.1[b]	0.46.0 ± 0.2[b]	0.53 ± 0.1[b]
Superoxide dismutase	36.0 ± 2.1	18.7 ± 5.9[a]	32.5 ± 3.9[b]	35.2 ± 3.7[b]	30.8 ± 5.1[ns]
Catalase	56.9 ± 0.6	45.4 ± 0.3[a]	65.2 ± 0.3[b]	71.7 ± 0.2[b]	61.2 ± 0.1[b]
Vitamin E	152.7 ± 3.5	82.7 ± 2.1[a]	138.7 ± 12.0[b]	148.5 ± 6.7[b]	127.2 ± 6.2[b]

Values are expressed in mean ± S.D; [a]p= Significant different from Group I vs. Group II ($p<0.001$); [b]p= Significant different from Group II vs. Group III, IV and V ($p<0.001$)

Discussion

Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders in ethno medical practices as well as in traditional systems of medicine in India (Bhattacharya et al., 2000). Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Hence there is worldwide trend to go back to traditional medicinal plants. Many natural products of natural origin are used for treatment of liver ailments (Pullareddy and Lokesh, 1996). Toxicity is mostly depen -dant on iron induced free radical reactions and oxidetive injury. Main pathophysiological effects of iron overload on liver tissue or fibrosis, porphyria and hepatocellular carcinoma. Free radical generation and lipid per oxidation is the proposed mechanism of iron induced hepatotoxicity. Iron catalyses hydroxyl radical formation and initiate lipid per oxidation (Reilly et al., 1991).

In the present study the level of glucose and bilirubin were significantly increased in ferrous sulfate induced groups when compared to control group of rats. Emerging scientific evidence has disclosed and suspected influences between iron metabolism and glucose level. The relationship is bidirectional iron affects glucose metabolism and glucose metabolism impinges on several iron metabolic pathways (Kirsch et al., 1968). Oxidative stress and inflammatory cytokines influence this relationship, amplifying and potentiating the initiated events. In recent years, increased iron stores have been found to predict the development of increased glucose levels while iron depletion was protective against beta cells of langerhan. Urinary bilirubin is a more sensitive indicator of liver injury than serum bilirubin (Hemalatha et al., 2005). The degree of increase in serum bilirubin values has prognostic significant in chronic liver injuries but not in acute injuries.

Iron hepatotoxicity, resulted in reduction of serum total protein and albumin levels in the present study. This observation could be ascribed to changes in protein and free amino acid metabolism and their synthesis in the injured liver cells and or increased protein degradation (Ratnasooriya et al., 2005). In our study the level of lipid profile via total cholesterol, triglycerides, LDL and VLDL was significantly elevated in ferrous sulfate induced group when compared to control group of rats where as the level of HDL was found to be significantly decreased. Our data indicate that dietary intake of herbal drugs and silymarin can significantly lower the serum lipid profile levels and can significantly increase HDL levels. It can also decrease endothelial cellular surface damage, rupture and may partially repair the endothelial dysfunction resulting from hyperlipidimia (Ramanathan and Kittusamy, 2011).

Iron poison is associated primarily with necrosis of the periportal areas, the site of hepatic regeneration. The periportal area receives the blood which is rich in oxygen and iron, both of which substrates for free radical generation. Iron catalyses the hydroxyl radical formation, the hydroxyl ion attacks all biological molecules, including cell membrane lipids, to initiate lipid per oxidation. The highly toxic peroxidative metabolite induces widespread cellular injury. Hepatic injury resulted in the leakage of cellular enzyme (alkaline phosphatase) into the blood stream, resulting in the augmented levels of serum enzyme (Kaplan, 1986). Serum levels of this enzyme are excellent indicator of hepatic parenchymal damage and dysfunction.

Oxidative stress is a common pathogenic mechanism contributing to initiation and progression of hepatic damage in a variety of liver disorders. Cell damage occurs when there is an excess of reactive species derived from oxygen and nitrogen or a defect of antioxidant molecule (Akhtar, 2013). In the present study significant decrease in the activity of liver super oxide dismutase, catalase and vitamin E in iron induced rats were observed which may be due to increased reactive oxygen species generation.

The rat liver was dissected out and fixed in 10% formalin and further processed for histopathological investigations. Histopathology of the liver of control group showed normal hepatic cells were as administration of ferrous sulfate in intoxicated group showed lesion with congestion and sign of necrosis Treatment with silimarin showed normal histological appearance

with regenerative changes and no sign of necrosis. *A. tetracantha* treated group showed slight evidence of necrosis and the overall architecture was near normal. *T. terrestris* treated group showed no infiltration and no necrosis. Thus the result of histopathology of the liver further confirmed the hepatoprotective activity of hydroalcoholic extract of *A. tetracantha* and *T. terrestris*.

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